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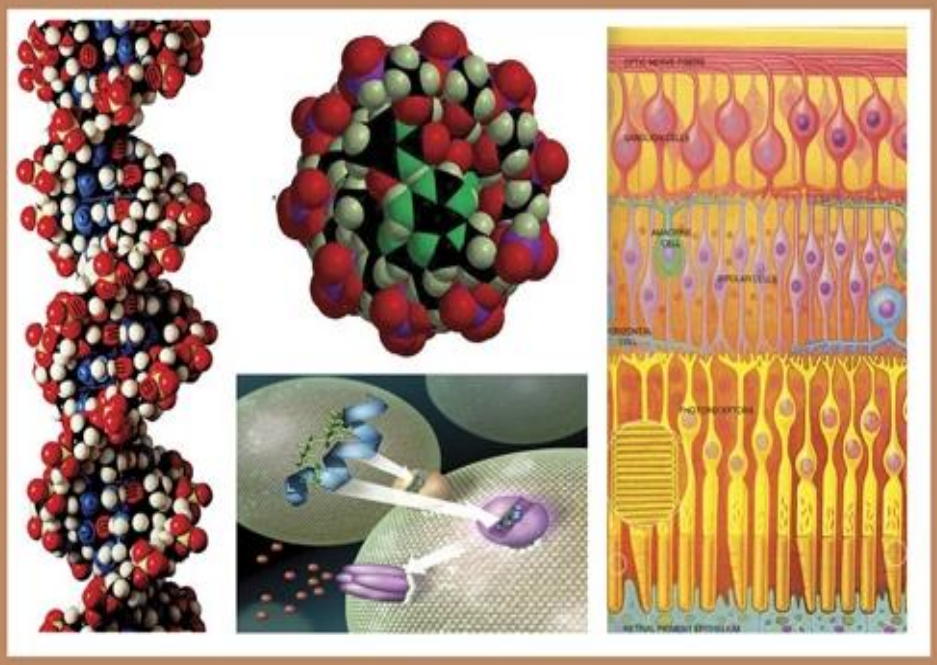

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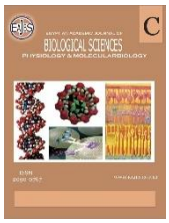

Novel Study of Testicular Volumes and Reproductive Hormones are Associated with the Infertility in Men with Non-Obstructive Azoospermia

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ABSTRACT

The purpose of this study is to assess a potential association between reproductive hormone levels and testicular volumes with testicular biopsy in men with non-obstructive azoospermia (NOA) undergoing testicular sperm extraction (TESE). The complete records of 100 azoospermia men without clinical evidence of obstructive etiologies who underwent TESA. Preoperative diagnostic biopsies were not obtained and thus men with presumed NOA were defined as having no evidence of obstruction by history or physical examination. Preoperative FSH, LH and testosterone were obtained for all patients and bilateral testicular volume was determined to find the correlation between probabilities of positive sperm extraction with TESE. Two subgroups of NOA were evaluated (Group 1 TESE and Group 2 Fresh ejaculated). Results: There were significant differences in FSH, levels, in group 2 compared to group 1, and no significant differences in LH, PRL, Testosterone, and E2. Furthermore, no significant differences in right testicular volume, and left testicular volume in the two groups. The success rate of TESE was decreased when increased FSH and LH levels. In NOA cases increased FSH and LH levels, and decreased testis volumes have a negative effect on sperm retrieval but small testicular volumes only can present sperm retrieval.

INTRODUCTION

Testes are the most important male reproductive organs which are responsible for the production of sperm (spermatogenesis) and hormones required for the development and maintenance of male sexual characteristics. Any disturbances in spermatogenesis and hormones related to oligozoospermia, athienozoospermia, azoospermia and lead to male infertility.Infertility is present in at least 30% of men internationally, and azoospermia accounts for 10% to 15% of male infertility (Agarwal *et al*., 2015; Jarow *et al*., 1989). Azoospermia, defined as the complete absence of ejaculated sperm, is the most severe form of infertility. It can be roughly divided into obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). In OA, testicular spermatogenesis function is preserved, and azoospermia is caused by the mechanical obstruction of any region along the reproductive tract. In NOA, testicular defects are present, and sperm production is remarkably impaired (Achermann *et al*., 2021).

Non-obstructive azoospermia (NOA) is a major cause of male infertility, with a prevalence of about 1% of the male population (Su *et al*., 1999). Patients with NOA have no spermatozoa in their semen because of impaired spermatogenesis in the testes (Turunc *et al*., 2010). However, sperm production can reportedly be detected in the testes of nearly 60% of men with NOA (Schlegel, 2009). Some techniques have recently been used to obtain sperm from these patients, such as testicular sperm extraction (TESE), fine needle aspiration (FNA), and microdissection TESE; these techniques could effectively treat male infertility combined with intracytoplasmic sperm injection (ICSI), (Devroey *et al*., 1995).

The combination of an elevated serum follicle stimulating hormone (FSH) level of more than 7.6 IU/L and smaller testicular volumes with a long axis of 4.6 cm or less predicts the etiology of azoospermia being due to spermatogenic dysfunction (Schoor *et al*., 2002). This has led to testis biopsy rarely being indicated in the diagnostic assessment to differentiate between obstructive azoospermia and NOA. However, it is common practice for a testicular biopsy to be obtained for the permanent sector at the time of micro TESE to help define the testicular histopathology and the severity of the testicular dysfunction.

Testicular biopsy is testicular sperm aspiration (TESA), which was initially used as a diagnostic tool in the assessment of azoospermia. If performed accurately, TESA can be used to sample areas of spermatogenesis that may be missed with a simple open biopsy (TESE). The technique enables the surgeon to reach broad areas within the testicle (Nowroozi *et al*., 2012). TESA can be done with testicular mapping in which the testicle is divided into a grid and aspiration is taken through each grid with separate punctures in even distributions (Beliveau and Turek, 2011).

In general, there is an inverse relationship between FSH levels and spermatogonia quantity (Ishikawa *et al*., 2004; Matin-du-Pan and Bischof, 1995). When spermatogonia number is absent or extremely reduced, FSH levels increase; when spermatogonia number is normal, FSH levels are within normal ranges. FSH levels also relate to the proportion of seminiferous tubules exhibiting Sertoli cells only on testicular biopsies (Bergmann *et al*., 1994).

Testicular size, texture, and consistency should be assessed. In routine practice, testicular volume is estimated using Prader's orchidometer. The mean testicular volume measured using the Prader's orchidometer in the general population is 20.0 mL (Boeri *et al*., 2021).So, the aim of the study was to assess the accuracy of different factors in predicting the sperm retrieval rate in patients with NOA. The study widely investigated predictive factors, including plasma FSH, LH, testosterone level and testicular volume.

MATERIALS AND METHODS

The study population consisted of 100 couples who were referred to assisted reproduction at the Fertility Clinic at the International Islamic Center for Population Studies and Researches, Al-Azhar University, Cairo, Egypt. The 100 male subjects were divided into 2 groups according to semen parameters (Each group formed of 50 cases), group 1, called testicular biopsy (TESE), and fresh semen, group 2.

Complete Semen Analysis:

Semen samples were collected by masturbation after a 3 to7 day period of sexual abstinence. Physical examinations including volume, color, odor, and liquefaction were done.

Microscopic examination was done to evaluate sperm (If present) concentration, motility, morphology, and the presence of another cellular element by light microscope (Olympus, C 21- Japan).

Sperms were classified into progressive motile, non-progressive and immotile.

Medical History:

Thorough medical history is critical to help determine the type of azoospermia. It must cover eight critical elements which are:

1. Infertility history.
2. Sexual history.
3. Childhood and development history.
4. Personal medical history.
5. Previous surgery/treatments.
6. Gonadotoxic exposure.
7. Family history.
8. Current health status and lifestyle.

Physical Examination:

The physical exam is critical in the assessment of men present with azoospermia. Testicular size, texture, and consistency should be assessed. In routine practice, testicular volume is estimated using Prader's orchidometer. The mean testicular volume measured using the Prader's orchidometer in the general population is 20.0 ± 5.0 mL (Boeri *et al*., 2021).

Hormonal Evaluation:

Assessment of reproductive hormones serum levels may add significant information to establish azoospermia type. Follicle-stimulating hormone (FSH) and testosterone are the essential hormones driving spermatogenesis (Esteves, 2015; Esteves, 2012). Prolactin and estradiol hormones. Testosterone is produced by the Leydig cells under luteinizing hormone (LH) stimulation. Adequate levels of intratesticular testosterone are critical for sperm maturation (Shiraishi, *et al*., 2012). By contrast, FSH is mainly responsible for increasing sperm production, and it collaborates with intratesticular testosterone to promote cell proliferation (Oduwole *et al*., 2018).

Selection Criteria:

The present studies included the following criteria:

(1) Patients diagnosed with NOA; (2) Patients not treated with hormone drugs before the operation; (3) Patients who underwent TESE.

Surgical Procedure:

All patients underwent surgery according to the same algorithm and surgical technique described by Silber *et al.,* (1996). The surgical intervention was performed under spinal anesthesia. The tunica vaginalis is opened following a midline scrotal incision. The testis is opened widely in an equatorial plane in the middle, revealing the testis covered with the tunica albuginea. As a result, seminiferous tubules can be exposed widely in a natural manner that mimics intratesticular blood flow. The remaining steps of the operation are carried out under a 20–25x operating microscope.

The tubules are removed for small samples. Sperm are more likely to be found in bigger and more opaque tubules. Depending on the size of the testicles and the condition of the tubules, up to 15 biopsies may be collected from each side. Once all visible parenchymal regions have been examined under a microscope or when additional dissection is deemed likely to endanger the testicular blood supply, the surgery is over.

Sample intended for the preliminary investigation of testicular tissue. After the initial samples were taken, the tissue was examined intra-operatively, and a preliminary assessment of the presence or lack of spermatozoa in the samples was made.

Before centrifugation, the tissue was mechanically macerated and suspended in the washing solution (Sperm Air, Ginemed). The sediment was inspected under a phase contrast microscope at x200 magnification. The outcome was reported intraoperatively. If no sperm were discovered, the tissue was then exposed to the enzymatic lysis procedure. The collagenase solution and tissue suspension were combined in a 1:1 ratio, and the mixture was shaken every 10–15 minutes throughout an incubation period of 60 minutes at 37°C. After the incubation, the undigested tissue was pelleted, and the

supernatant was separated using centrifugation at 50 g for 5 minutes. Enzymes were taken out by adding an equivalent amount of wash media. The supernatant was separated using two 1800 g/5-minute centrifugations to separate the sample. The sediment was checked with a micro drop on the day of the intervention, and the results were recorded.

Statistical Analysis:

Data were coded and entered using the statistical package for (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. For comparing categorical data, P-values less than 0.05 were considered statistically significant.

RESULTS

Table (1) shows the patient data distribution in the TESE group with ages ranging from (20 to 48) years old, mean ejaculated volume of 1.9 ml while the recorded sperm count mean at 0.00 before testicular biopsy and 0.002 after TESE, (Fig 1).

Table (2) shows the patient data distribution with mean hormonal value for FSH (13.4) and fluctuating between (0.3 to 39 IU/L), luteinizing hormone (9.1) and ranging between (0.3 to 20 IU/L), PRL (14.7) and ranging between (4.9 to 40.9 IU/L), free testosterone (7.12) and ranging between (0.6 to 25.9 IU/L), total testosterone (4.6) and ranging between (0.8 to 14.7 IU/L), finally E2 (35.4) and ranging between (17.5 to 60 pg/ml), the calculated mean value for right testicular length is 3.9 cm, left testicular length is 3.8 cm, and left testicular width is 2.14 cm, right testicular width is 2.2 cm, (Table 2 and Fig 2).

Table (3) shows the patient data distribution in ejaculated semen group with ages ranging from (18 to 47) years old, mean ejaculated volume 2.6 ml while the recorded sperm count means 229.9 thousand, (Fig 3).

Table (4) shows the patient data distribution with mean hormonal value for FSH (9.3) and fluctuated between (1.5 to 33 IU/L), luteinizing hormone (5.8) and ranging between (2.5 to 11.6 IU/L), PRL (12.6) and ranging between (5.7 to 19 IU/L), free testosterone (8.03) and ranging between (2.8 to 25.5IU/L), total testosterone (4.6) and ranging between (1.2 to 17.3IU/L), finally E2 (36.5) and ranging between (25.6 to 50 pg/ml), the calculated mean value for right testicular length is 3.9 cm, left testicular length is 3.9 cm, and left testicular width is 2.2 cm, right testicular width is 2.3 cm, (Table 4 and Fig 4).

Table (5) and Figures 5&6, shows the patient data distribution between the two groups, mean hormonal value for FSH, showed a significant difference in ejaculated semen groups compared to TESE group ($p<0.05$) and insignificant difference in luteinizing hormone, free testosterone, total testosterone and, E2 which compared with TESE group. The mean value for the right testicular's length, left testicular's length, left testicular's width, and right testicular's width, showed although insignificant difference ($p>0.05$).

Table 1: Patient data distribution in TESE group.

	Age	Volume ml	Sperm count	Positive TESE
Mean	33.56	1.9	0.00	0.002
St.dev.	7.9	0.82	0.00	0.001
Minimum	20	0.3	0.00	0.00
Maximum	48	3.9	0.00	0.008

(St.dev.) stander deviation; (TESE) testicular sperm extraction**.**

Table 2: Patient data hormones and testicular volume, mean, std, and range for patients.

	FSH	LH	PRL	Free Testosterone	Total Testosterone	E2	Testicular length		Testicular Width	
							L	R	L	R
Mean	13.4	9.1	14.7	7.12	4.56	35.4	3.8	3.9	2.14	2.2
St.dev.	8.8	4.3	8.04	4.9	3.54	8.7	0.64	0.60	0.33	0.33
Minimum	0.3	0.3	4.9	0.6	0.8	17.5	2.2	2.7	1.1	1.5
Maximum	39	20	40.9	25.9	14.7	60	4.8	4.9	2.8	2.8

(FSH) Follicle-stimulating hormone; (LH) luteinizing hormone; (PRL) Prolactin; (E2) estradiol hormones; (St.dev.) stander deviation.

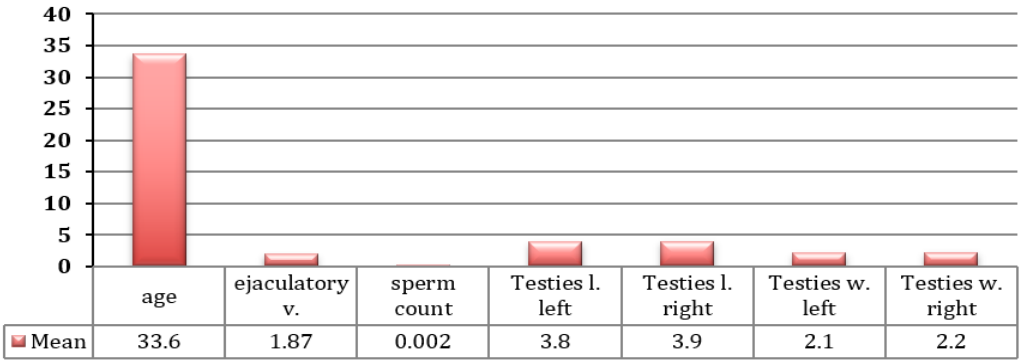


Fig. 1: Data distribution in TESE group

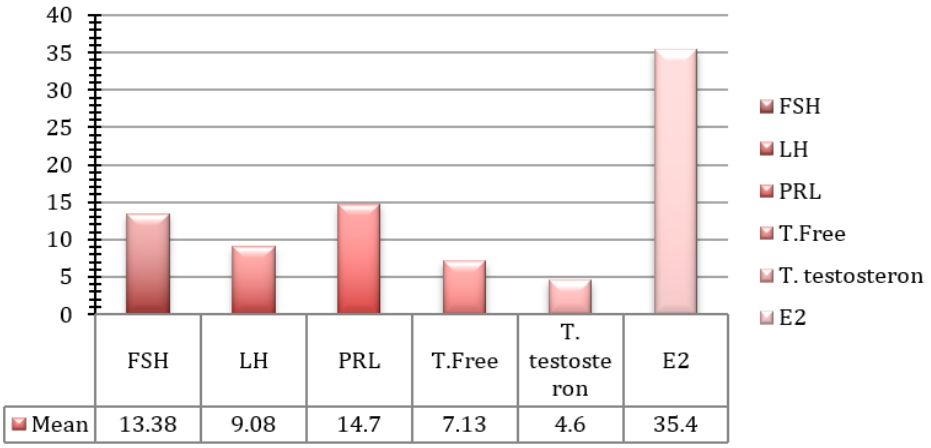


Fig. 2: Patient data mean hormones and testicular volume.

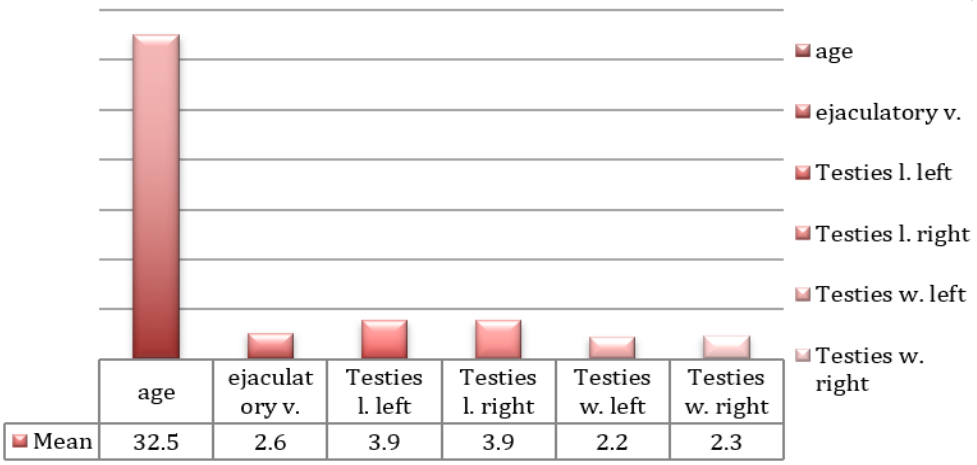


Fig. 3: Data distribution in TESE group ejaculated semen group

Table 3: Patient data distribution in ejaculated semen group.

	Age	Volume ml	Sperm count/ thousand
Mean	32.52	2.63	229.9
St.dev.	7.8	0.7	202.01
Minimum	18	0.6	0.0001
Maximum	47	3.9	1000

(St.dev.) stander deviation

Table 4: Patient data hormones and testicular volume, mean, std, and range for patients

	FSH	LH	PRL	Free Testosterone	Total Testosterone	E2	Testicular length		Testicular Width	
							L	R	L	R
Mean	9.3	5.8	12.6	8.03	4.62	36.5	3.9	3.9	2.2	2.3
St.dev.	6.6	2.3	3.2	5.8	3.7	6.9	0.4	0.6	0.4	0.44
Minimum	1.5	2.5	5.7	2.8	1.2	25.6	3.1	2.0	1.7	1.7
Maximum	33	11.6	19	25.5	17.3	50	4.6	4.9	4.3	4.5

(St.dev.) stander deviation; (FSH) Follicle stimulating hormone; (LH) luteinizing hormone; (PRL) Prolactin; (E2) estradiol hormones.

Table 5: Patient data hormones and testicular volume between two groups**.**

	FSH	LH	PRL	Free Testosterone	Total Testosterone	E2	Testicular length		Testicular Width	
							L	R	L	R
Biopsy	13.4	9.1	14.7	7.12	4.56	35.4	3.8	3.9	2.14	2.2
Semen ejaculated	9.3	5.8	12.6	8.03	4.62	36.5	3.9	3.9	2.2	2.3
P value	0.01*	0.47	0.088	0.40	0.92	0.49	0.28	0.49	0.78	0.24

(FSH) Follicle-stimulating hormone; (LH) luteinizing hormone; (PRL) Prolactin; (E2) estradiol hormones. P-values less than 0.05 were considered statistically significant.

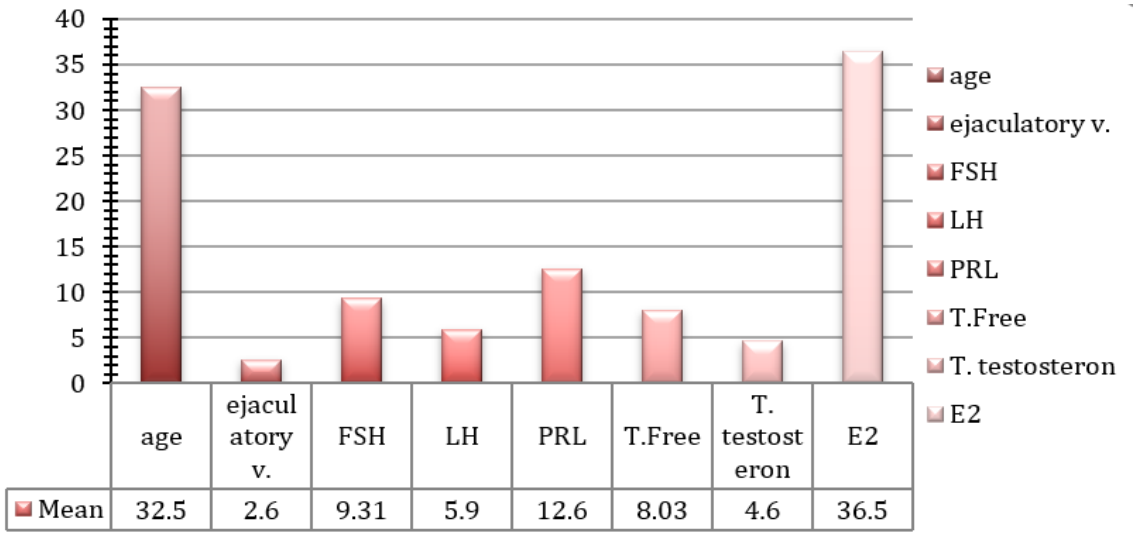
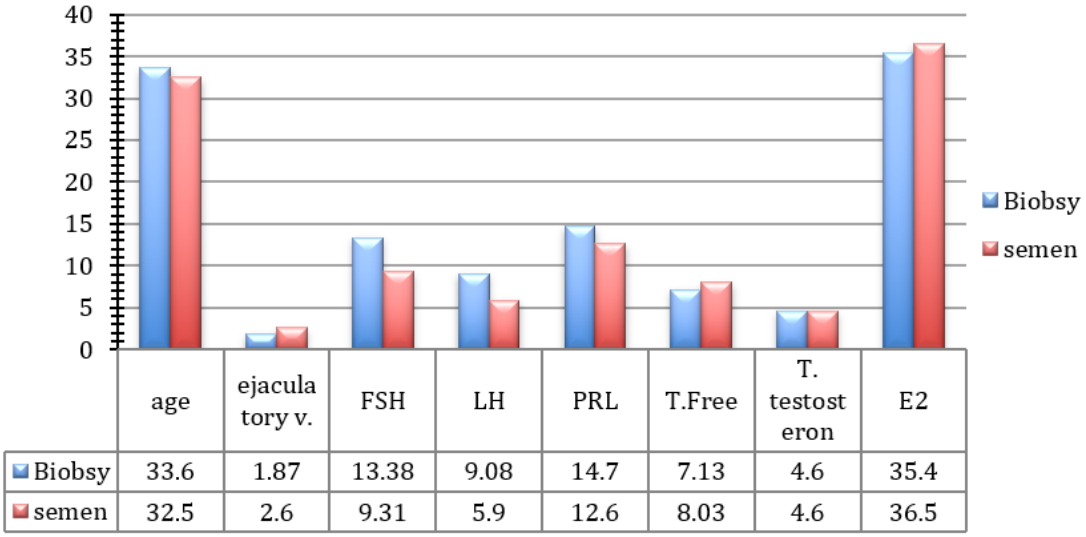
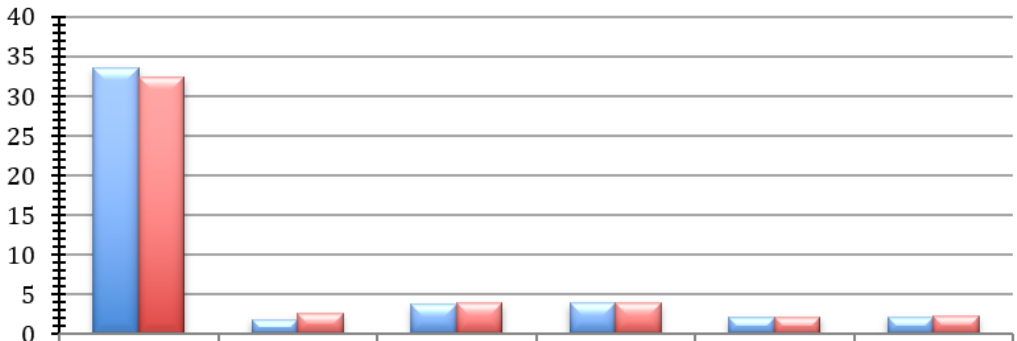


Fig. 4 : Patient data mean hormones and testicular volume.



	age	ejaculatory v.	FSH	LH	PRL	T.Free	T. testosteron	E2
Biobsy	33.6	1.87	13.38	9.08	14.7	7.13	4.6	35.4
semen	32.5	2.6	9.31	5.9	12.6	8.03	4.6	36.5

Fig. 5: Patient data distribution of hormones between two groups.



	age	ejaculatory v.	Testies l. left	Testies l. right	Testies w. left	Testies w. right
Biobsy	33.6	1.87	3.8	3.9	2.1	2.2
semen	32.5	2.6	3.9	3.9	2.2	2.3

Fig. 6: Showing testicular volumes between two groups.

DISCUSSION

Azoospermia is diagnosed in approximately 20% of infertile men, whereas patients with NOA represent 15% of all infertile men (Palermo *et al*., 1999; Turek *et al*., 1999; Stanwell Smith *et al*., 1984). NOA is considered the most challenging situation in a couple's fertility care and essentials a high level of treatment for patients.

The differentiation between azoospermia due to obstruction and NOA due to spermatogenic dysfunction previously required a diagnostic testicular biopsy to assess the stage of spermatogenesis former to contribute definitive therapeutic options.

The present study showed increased levels of FSH with small testicular volume and the result is consistent with some studies, the clinical result of an increased serum follicle-stimulating hormone (FSH) higher than 7.6 IU/L and smaller volume testicles with a long axis of 4.6 cm or less has been conventional to expect the etiology of azoospermia to be due to spermatogenic arrest, or NOA (Schoor *et al*., 2002). Serum FSH levels and testicular volumes are among the most studied parameters. Increased FSH levels and small testicular volumes have been thought to be associated with testicular maturation arrest or testicular failure and FSH levels have been

shown to increase with the decreasing spermatogonia number. Moreover, spermatogonia production is known to occur even at very high serum FSH levels (Tournaye *et al*., 1997; Ishikawa *et al*., 2004). And increased serum FSH levels and smaller testicular volumes are associated with more severe testicular histopathology in men with NOA (Parviz *et al*., 2021).

Normal serum FSH levels do not indicate that the spermatogonia count is within normal limits and does not rule out spermatogenesis defects. Furthermore, sperm retrieval is possible in the presence of high serum FSH levels (Rowe *et al*., 2000).

The present result disagrees with the study by (Schwarzer *et al*., 2003) which concluded that there is no statistical correlation between serum FSH levels and sperm retrieval rates with the TESE procedure. Unlike these studies, serum FSH levels were found to have an important role in predicting sperm retrieval success in the present study.

Serum LH values fluctuate in patients with NOA. In the present study, the serum LH levels were found to be closer to normal values. However, the serum LH values of the TESE-negative patients can be higher. However, normal levels in cases where sperm cannot be obtained because the serum LH level is not to be used as a definite marker to predict sperm retrieval. Preoperative serum FSH level of azoospermia men has long been investigated as a prognostic indicator to correlate with TESA outcomes. Dajani found a strong positive correlation between serum FSH and the presence of mature sperm obtained via TESA when FSH was <10 IU/l (Dajani and Kilani, 1998).

In light of the present data, serum LH level alone is insufficient to expect sperm retrieval success and the result is in agreement with the study by Guneri *et al*., serum LH levels were found to have no statistically significant relationship with sperm retrieval achievement, but the increase in LH and FSH levels was observed to be correlated (Guneri *et al*., 2016). Pathologies that increase serum FSH levels also increase serum LH levels through a related mechanism, and therefore, serum LH level accompanies the high serum FSH levels, particularly in cases where sperm retrieval is unsuccessful. However, the results of previous studies on levels of hormones such as FSH, LH, and estradiol (E2) are controversial. Many researchers have proposed FSH as predictive of positive SRR (Ishikawa, 2012), while other authors disagree (Jezek *et al*., 1998; Li *et al*., 2018). In NOA cases increased FSH and LH levels, and decreased testis volumes have a negative effect on sperm retrieval (Barlas *et al*. 2021).

In the present study, Testicular volume showed an insignificant difference between the two groups and the result disagrees with the author (Corona *et al*., 2019) which stated that testis volumes (left and right) were the only significant prognostic markers for successful SRR found among several clinical and biochemical parameters.

In recent years, the development of an efficient technique for testicular sperm cryopreservation has played a crucial role in the preparations for ICSI that occur prior to oocyte collection, with the aim of providing for further treatment or a repeated cycle after an initial mTESE ICSI cycle with fresh sperm, thus avoiding repeated surgery. Therefore, many researchers have focused on estimating the clinical outcomes of fresh versus cryopreserved testicular sperm in ICSI. Some studies have reported similar results between the two groups (Kanto *et al*., 2015; Eken and Gulec, 2018), but some have suggested that fresh sperm yields better clinical outcomes (Zhang *et al*., 2021; Xiaoming Sun *et al.*, 2023). Testicular volume is another parameter that was widely investigated for predicting sperm retrieval. The testicular volumes of men with NOA were reportedly usually less than those of men with obstructive azoospermia.

In addition; the study of (Ziaee *et al*. 2006) showed that in patients with NOA, the average testicular volume was17.5 ml in men with positive sperm retrieval and 5.7 ml in men without sperm retrieval. This might indicate that smaller testicular volume was related to more severe spermatogenesis impairment. However, there might still be areas with normal spermatogenesis, even in a small testis. (Bryson *et al.* 2014) suggested that small testes should not be a contraindication for micro-dissection TESE in patients with NOA. Regardless of being unilateral or bilateral, the decrease in testicular volume is associated with the spermatogenesis defect. The fact that testicular volume is generally below 15mL in patients with NOA also supports this information. Patients with a testicular volume below 5mL should be carefully examined in terms of karyotypic disorders or pathologies that may cause azoospermia (Lipshultz and Corriere, 1977).

Conclousion

The success rate of TESE was decreased when increased FSH and LH levels. In NOA cases increased FSH and LH levels, and decreased testis volumes have a negative effect on sperm retrieval but small testicular volumes only can present sperm retrieval.

Ethical Approval:

This study was performed in accordance with the ethical committee of Al-Azhar University, Egypt.

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